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Award Number: W81XWH-06-1-0236

TITLE: Alcohol Intoxication Impact on Outcome from Traumatic Injury

PRINCIPAL INVESTIGATOR: Patricia Molina, M.D., Ph.D.

CONTRACTING ORGANIZATION: Louisiana State University
New Orleans, LA 70112

REPORT DATE: May 2009

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
1 May 2009	Annual	3 APR 2008 - 2 APR 2009

4. TITLE AND SUBTITLE
Alcohol Intoxication Impact on Outcome from Traumatic Injury

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0236

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Patricia Molina, M.D., Ph.D.

Email: pmolin@lsuhsc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Louisiana State University
New Orleans, LA 70112

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Acute alcohol intoxication (AAI) impairs the hemodynamic counteregulatory response to trauma and hemorrhagic shock (HS), blunts the pressor response to fluid resuscitation (FR), suppresses the HS-induced neuroendocrine response, impairs pro-inflammatory cytokine expression and increases mortality from infection during recovery. Studies conducted during this funding period examined a) whether the attenuated neuroendocrine response, particularly reduced sympathetic nervous system (SNS) activation, is the principal mechanism responsible for the hemodynamic instability seen in AAI+ HS and b) what the impact of AAI was on the integrity of host defense mechanisms during the immediate and delayed recovery from HS. We determined whether SNS activation can be restored by central (intracerebroventricular; ICV) neostigmine administration and whether this in turn is capable of improving the hemodynamic counteregulatory response to HS in AAI. Our results show that ICV neostigmine stimulates SNS activation and improves the recovery of blood pressure following hemorrhagic shock. Furthermore, our results indicate that this in part mediated by arginine vasopressin. Interestingly while the presser response to phenylephrine in vitro appears to be blunted by alcohol, the in vivo response to a presser with a different mechanism of action appears to be preserved.

15. SUBJECT TERMS
Alcohol intoxication, hemorrhage, injury, blood pressure, immune function

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	11	19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION: Acute alcohol intoxication (AAI) impairs the hemodynamic counteregulatory response to trauma and hemorrhagic shock (HS), blunts the pressor response to fluid resuscitation (FR), suppresses the HS-induced neuroendocrine response, impairs pro-inflammatory cytokine expression and increases mortality from infection during recovery. Studies conducted during this funding period examined a) whether the attenuated neuroendocrine response, particularly reduced sympathetic nervous system (SNS) activation, is the principal mechanism responsible for the hemodynamic instability seen in AAI+ HS and b) if alterations in the inflammatory response following HS during AAI observed in the recovery period could be restored by improving blood pressure recovery during fluid resuscitation. We determined whether SNS activation can be restored by central cholinergic activation and whether this in turn is capable of improving the hemodynamic counterregulatory response to HS in AAI. Our results show that ICV neostigmine stimulates SNS activation and improves the recovery of blood pressure and catecholamine response following hemorrhagic shock. Translational studies utilizing intravenous physostigmine have demonstrated improvements in SNS activation and blood pressure recovery, attenuated tissue injury, and partial restoration of the inflammatory response. Findings from additional vascular studies suggest that the impaired hemodynamic counterregulation during HS in AAI is not due to decreased vasopressor responsiveness. Together, these findings suggest central alterations in the sympathetic nervous system are responsible for the alcohol-induced impairment in neuroendocrine and hemodynamic responses to blood loss and provide the basis for a new therapeutic target in alcohol intoxicated trauma victims.

Progress report 3rd funding period:

Similar to last year, we have performed studies that fall under the 3 objectives of the proposal. Progress made in these will be described accordingly.

Objective 1: To test the hypothesis that acute alcohol intoxication alters central activation of descending sympathetic outflow. The proposed studies will identify the mechanisms responsible for the impaired hemodynamic counterregulatory response to blood loss in the alcohol-intoxicated host. Specifically, to isolate central and peripheral regulatory mechanisms disrupted during alcohol intoxication.

a. Determine whether direct central activation of sympathetic outflow restores catecholaminergic and hemodynamic responses to hemorrhagic shock in alcohol-intoxicated animals.
b. Determine whether inhibition of central sympathetic activation during alcohol intoxication is mediated through enhanced tonic inhibition by nitric oxide.
c. Examine whether central administration of arginine vasopressin enhances sympathetic activation and restores catecholaminergic and hemodynamic responses to hemorrhagic shock in alcohol-intoxicated animals.

Progress: Research Objective 1a

Central neostigmine administration reverses alcohol- and hemorrhage-induced hypotension. One of the most critical determinants of outcome within the first 48 hours of injury is the victim's mean arterial blood pressure (MABP) at the time of admittance into the emergency department. Previously we have demonstrated that ICV choline increased basal MABP (+17%) and produced a similar increase in basal MABP in alcohol intoxicated. However, ICV choline did not alter the initial % decrease in blood pressure nor did it improve MABP throughout hemorrhagic shock or fluid resuscitation in alcohol-treated animals. These studies showed that intracerebroventricular (ICV) choline (acetylcholine precursor) administration produced a transient activation of sympathetic nervous system outflow insufficient to improve MABP following AAI + HEM. In more recent studies, we have demonstrated that ICV neostigmine administered 10 minutes after the initiation of hemorrhage reversed the 12% (P=0.001) drop in MABP caused by alcohol

(ALC; 2.5 g/kg, 30% v/v) administration within 30 min, completely reversed the hypotension produced by 40% total blood loss, and improved 7-day survival (100% vs. 25%) from HEM. These results demonstrate that ICV neostigmine reverses hemorrhage- and alcohol-induced hypotension and produces a more sustained elevation in MABP.

In addition to an improved hemodynamic response, ICV neostigmine contributed to a restoration of the neuroendocrine response to blood loss. Hemorrhagic shock produced a marked 542% (P = 0.007) and 522% (P = 0.022) increase in circulating levels of epinephrine at the end of hemorrhage in dextrose controls and alcohol-treated animals, respectively. ICV neostigmine did not alter post-hemorrhage epinephrine levels in dextrose controls, but further enhanced the hemorrhage-induced increase in alcohol-treated animals (109%; P = 0.004). A significant increase in circulating norepinephrine (123%; P = 0.023) was observed at the end of hemorrhage in dextrose controls, which was prevented by alcohol. As with epinephrine, ICV neostigmine did not alter the hemorrhage-induced increase in plasma norepinephrine in dextrose controls, but restored the hemorrhage-induced increase in alcohol-treated animals. Hemorrhagic shock increased circulating levels of AVP by 125% (P = 0.064) in dextrose animals, which was also prevented by alcohol. ICV neostigmine restored the hemorrhage-induced increase in alcohol-treated animals.

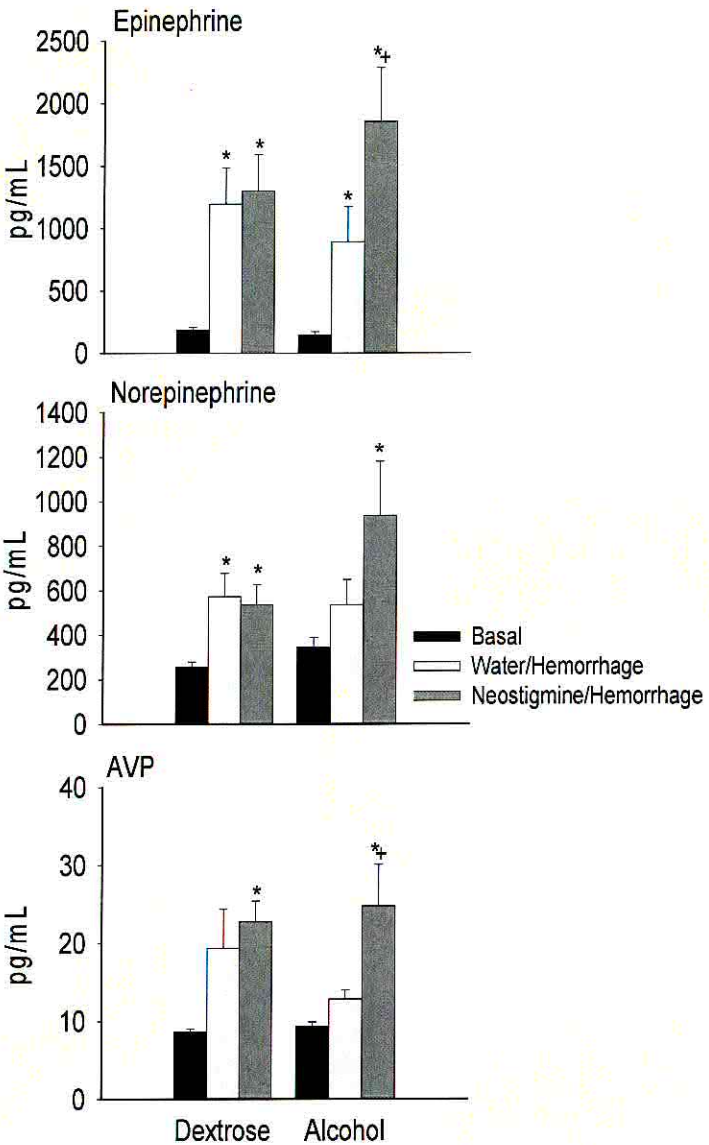

Fig 1. ICV neostigmine improves neuroendocrine response to hemorrhage during acute alcohol intoxication: Effects of neostigmine (0.5 g) or sterile water (5 L) on plasma epinephrine, norepinephrine and arginine vasopressin (AVP) in pg/mL in dextrose controls (left column) and alcohol-treated animals (right column) subjected to 50% hemorrhage and fluid resuscitation (n = 7-9). Data analyzed using two-way ANOVA with repeated measures. *P versus basal; +P versus water/hemorrhage group.

These findings suggest that the alterations in hemodynamic and neuroendocrine response to blood loss during AAI are the central in origin and can be restored through central cholinergic activation of descending sympathetic outflow.

Intravenous administration of physostigmine improves blood pressure recovery and decreases early tissue injury following HS during AAI.
Because central pharmacological interventions are not feasible or practical in the clinical setting, these studies examined the acetylcholinesterase inhibitor physostigmine, which can be administered intravenously and cross the blood-brain barrier to produce central effects. IV physostigmine produced similar stimulation of descending sympathetic nervous system outflow to that elicited by ICV neostigmine injection. Physostigmine increased MABP by 12% (P = 0.003) within five minutes. The pressor response

peaked at 15 minutes (21%; P < 0.001) and was diminished by 30 minutes. IV physostigmine administration produced an immediate (within 5-15 minutes) increase in heart rate (21%; P = 0.003) and circulating levels of epinephrine (358%; P = NS), norepinephrine (105%; P = NS), and glucose (26%; P = 0.005) but decreased plasma levels of insulin (47%; P = 0.002).

To examine the effects of peripheral physostigmine administration, chronically instrumented, conscious male Sprague-Dawley rats (300-350 g) received a primed continuous 15 hour intragastric ALC infusion (2.5g/kg + 300 mg/kg/hr) or isocaloric/isovolumic dextrose. Thirty minutes after discontinuing ALC, animals were subjected to fixed pressure hemorrhage (40 mmHg for 60 min) followed by fluid resuscitation consisting of a primed-constant infusion of Ringer's lactate (40% bolus + 2x total blood volume removed over 60 min). Animals were randomly assigned to receive intravenous physostigmine (100 ug/kg) or saline vehicle. Physostigmine significantly improved blood pressure recovery in both alcohol and dextrose treated groups. This improved hemodynamic recovery was associated with attenuated hepatic damage (ALT) and mortality during the recovery period. Ongoing studies are examining the ability of central cholinergic activation via physostigmine to improve MABP and survival when administration is delayed beyond the initial 60 minute hypotensive period. Early observations from these studies suggest that the effectiveness of physostigmine is not diminished when administration is delayed and its ability to improve outcome may become more critical as the intensity of the hemodynamic insult is increased.

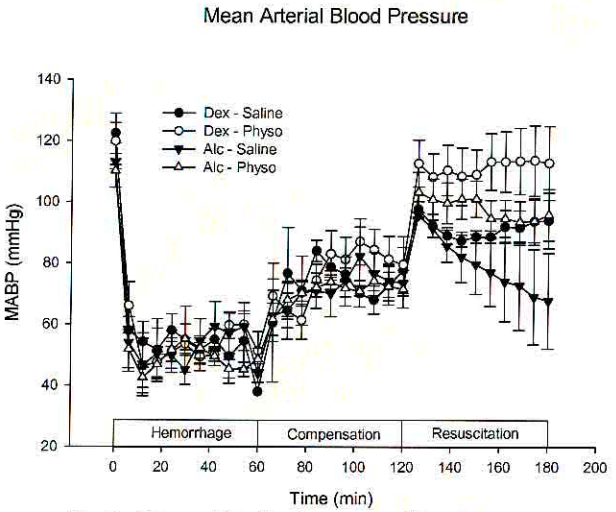

Fig 2. Physostigmine improves blood pressure recovery after delayed administration. Mean arterial blood pressure during fixed pressure hemorrhage, a 60 minute delay, and fluid resuscitation +/- physostigmine (n = 3-5).

Objective 2: Examine the impact of alcohol intoxication on vascular responsiveness to pressor agent administration.

a. To determine the impact of acute alcohol intoxication during trauma/hemorrhage on vascular responsiveness to in vivo administration of pressor agents (norepinephrine and arginine vasopressin).

b. To examine the impact of alcohol on vascular reactivity to direct application of pressor agents to isolated vessels.

Progress objective 2b

Vasodilatory, but not vasoconstrictive, response in isolated aortic and mesenteric rings is altered by hemorrhage. This study examined the effects of AAI + Hemorrhage/Resuscitation on blood vessel reactivity to phenylephrine (PE), acetylcholine (Ach), and nitroprusside (NP) ex-vivo. Chronically instrumented, conscious male Sprague-Dawley rats (300-350 g) received a primed-constant 15 hour intragastric ALC infusion (2.5g/kg + 300 mg/kg/hr) or isocaloric/isovolumic dextrose prior to fixed pressure

hemorrhage (40 mmHg for 60 min) followed by fluid resuscitation consisting of a primed-constant infusion of Ringer's lactate (40% bolus + 2x total blood volume removed over 60 min). Animals were sacrificed at the end of hemorrhage or resuscitation for isolation of thoracic aorta and mesenteric arteries. Aortic and mesenteric ring segments (1-2 mm) were suspended in myograph baths containing Krebs-Henseleit bicarbonate buffer, pH 7.4, gassed with 95% O2: 5% CO2.

Previous studies demonstrated arterial rings from AAI rats had decreased PE-induced tension (aorta: 2.28 ± 0.09 vs. 2.6 ± 0.13 g; mesenteric artery: 1.70 ± 0.06 vs. 1.91 ± 0.10), greater (21%) Ach-mediated relaxation, and similar NP-mediated relaxation. These results indicate that AAI favors vasodilatation and are consistent with enhanced endothelial dilator function .

This study examined the vasoconstrictive and vasodilatory responses in isolated aortic and mesenteric arteries following hemorrhage and fluid resuscitation in control and alcohol-intoxicated animals. In contrast to previous findings, AAI alone or in combination with hemorrhage +/- resuscitation failed to produce significant alterations in pressor or dilatory responses. Hemorrhage, however, was associated with increased dilatory response to multiple doses of both acetylcholine and sodium nitroprusside, indicating enhanced vasodilation via both endothelium-mediated and endothelium independent mechanisms respectively. These alterations persisted despite fluid resuscitation.

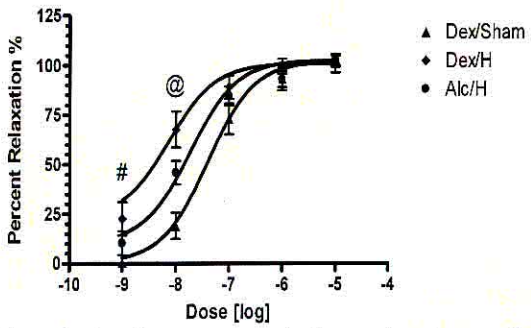

Fig 3. Mesenteric artery response to increasing doses of acetylcholine following hemorrhage. Percent relaxation was normalized to the Sham (non-infused, non-hemorrhaged) group in which the maximum dilation was set to 100% and dilation in the other groups compared to that maximum# $p < 0.05$ different from Dex/sham; @ $p < 0.01$ different from Dex/sham. Values are mean +/- SEM.

Taken together, these findings suggest that the impaired hemodynamic counterregulation to blood loss during AAI is not due to decreased vascular responsiveness and demonstrates a role for accentuated vasodilatory responses which may be central in the progression to decompensated shock.

Research Objective 3: To test the hypothesis that the alterations in hemodynamics produced by acute alcohol intoxication during trauma-hemorrhage result in inadequate tissue perfusion during the resuscitation period leading to enhanced susceptibility to tissue injury.

a. Examine the impact of acute alcohol intoxication on tissue blood flow redistribution following fluid resuscitation.

b. Identify the host defense mechanisms affected by alcohol intoxication & traumatic injury that impair the ability to effectively respond to a "second hit" infectious challenge.

Progress Objective 3b.

Improving blood pressure recovery leads to improved regulation of the inflammatory response following HS in AAI. Previously, we demonstrated that acute alcohol intoxication prior to hemorrhagic shock impairs hemodynamic and neuroendocrine counterregulation and increases mortality from infection during recovery. Additionally, alcohol-binge prior to hemorrhagic shock (HS) has been shown to suppress early pro-inflammatory cytokine expression, providing a possible mechanism for the increased mortality from infection during recovery. Recently, we have observed suppression of peripheral blood mononuclear

cells' (PBMC) pro-inflammatory cytokine response to a "second-hit" inflammatory challenge 1 day after Trauma-HS (TxHS). Follow-up studies examined the effects of alcohol-binge prior to TxHS on the immune responsiveness of PBMCs 5 days after injury. In contrast to findings at 1 day post-TxHS, an upregulation of the inflammatory cytokine response was observed later during the recovery period. These findings suggest an overall dysregulation of the inflammatory response, leading to increased susceptibility to infection during the early recovery period and enhanced organ damage during the late pro-inflammatory phase.

To further examine the mechanisms of the altered inflammatory response following HS during AAI, we examined the role of the greater hypotension observed in this group. Chronically instrumented, conscious male Sprague-Dawley rats (300-350 g) received a primed continuous 15 hour intragastric ALC infusion (2.5g/kg + 300 mg/kg/hr) or isocaloric/isovolumic dextrose prior to fixed pressure hemorrhage (40 mmHg for 60 min) followed by fluid resuscitation consisting of a primed-constant infusion of Ringer's lactate (40% bolus + 2x total blood volume removed over 60 min). Animals were randomly assigned to receive intravenous physostigmine (100 ug/kg) or saline vehicle at the time of fluid resuscitation. Physostigmine is an acetylcholinesterase inhibitor with similar mechanisms as the previously described neostigmine, but allows for peripheral administration and thus a more clinically relevant resuscitation model. Following resuscitation, animals were allowed 24 hours to recover prior to intra-arterial LPS (1 mg/kg). AAI prior to HS produced a significantly greater hypotensive response to LPS which was prevented by administration of physostigmine at the time of fluid resuscitation. LPS produced a robust increase in the circulating cytokines TNF-a and IL-6. AAI showed a trend to blunt the inflammatory response while HS accentuated the LPS-induced rise in TNF-a and IL-6. AAI at the time of hemorrhage blunted this hemorrhage-induced exaggerated response. Plasma IL-6, however, was partially recovered in animals that received physostigmine resuscitation. Additionally, pro-inflammatory cytokine content in the lung was reduced in AAI receiving physostigmine. These findings suggest that improved blood pressure recovery leads not only to an improved plasma inflammatory response, but better overall regulation of the inflammatory response.

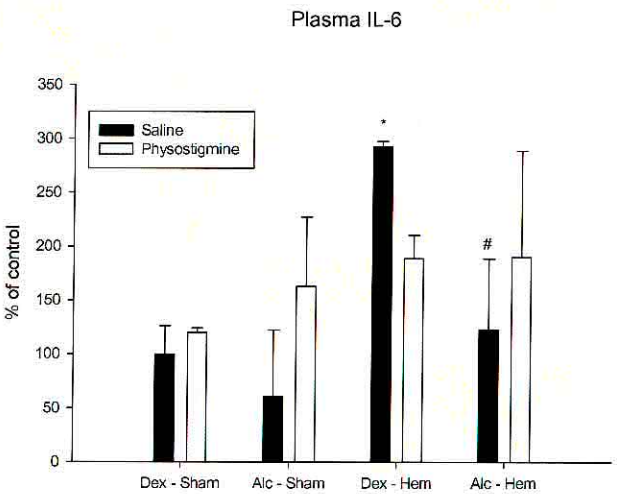

Fig 4. Physostigmine administration partially restores the inflammatory response to LPS 24 hours post-hemorrhage in AAI. IL-6 response 90 minutes post intra-arterial LPS (1 mg/kg) 24 hours after hemorrhage/resuscitation.
* p<.05 vs dex/sham.

Key research accomplishments:

- Demonstrated that neostigmine reverses hypotension produced by blood loss in control and alcohol-intoxicated animals
- Demonstrated that neostigmine produces significant increases in catecholamines in response to blood loss during AAI

- Demonstrated that AAI + hemorrhage accentuates the vascular vasodilatory response
- Demonstrated that improving blood pressure recovery following hemorrhagic shock leads to an improved pro-inflammatory cytokine response to a subsequent systemic challenge
- Demonstrated that central cholinergic activation plays a role in the dysregulation of the inflammatory response following hemorrhage in AAI

Reportable outcomes

Publications:

Mathis KW, Molina PE. Transient central cholinergic activation enhances sympathetic nervous system activity but does not improve hemorrhage induced hypotension in alcohol-intoxicated rodents. *Shock*. Feb 2 (Epub), 2009.

Molina, Miguel F, Annie Whitaker, Patricia E Molina, Kathleen H McDonough Alcohol does not Modulate the Augmented Acetylcholine-induced Vasodilatory Response in Hemorrhaged Rodents *Shock* 2009.

Mathis KW, Molina PE. Central acetylcholinesterase inhibition improves hemodynamic counterregulation following severe blood loss in alcohol-intoxicated rodents. *American Journal of Physiology: Regulatory and Comparative Physiology*. Submitted March 24, 2009.

Mathis KW, Molina PE. Systemic administration of an acetylcholinesterase inhibitor improves outcome and survival from severe hemorrhagic shock during acute alcohol intoxication. *Critical Care Medicine*. In preparation.

Presentations:

KW Mathis and PE Molina. Acetylcholinesterase inhibitor improves survival from hemorrhage in rodents. FASEB, New Orleans, April 2009.

KW Mathis and PE Molina. Alcohol-induced hemodynamic instability during hemorrhagic shock is reversed by acetylcholinesterase inhibitor. Alcoholism: Clinical and Experimental Research, Washington DC, July 2008.

KW Mathis, AM Whitaker, PE Molina. Effective mechanisms in restoring blood pressure following hemorrhage. FASEB, San Diego, CA, April 2008.

KW Mathis and PE Molina. Central neostigmine administration reverses alcohol- and hemorrhage-induced hypotension. FASEB, San Diego, CA, April 2008.

AM Whitaker, JK Sulzer, PE Molina. The impact of acute alcohol intoxication on vascular responsiveness to in vivo administration of arginine vasopressin following hemorrhagic shock. Research Society SA Washington DC June 2008.

JK Sulzer and PE Molina. Increasing blood pressure during resuscitation from hemorrhagic shock partially restores the inflammatory response during recovery in alcohol intoxicated rats. Gulf Coast Physiological Society Meeting, New Orleans, La, April 2009.

C Hakenjos, JK Sulzer, PE Molina. Effects of increasing blood pressure during resuscitation from hemorrhagic shock on the pulmonary inflammatory response during recovery in acute alcohol intoxicated rats. School of Allied Health Professionals Research Day, LSUHSC, New Orleans, La, April 2009.

Conclusions:

The results from our ongoing studies have provided evidence that central cholinergic stimulation may be an effective intervention to enhance sympathetic outflow in alcohol-intoxicated hemorrhaged animals. Our most recent findings from those studies have shown that central administration of the acetylcholinesterase inhibitor, neostigmine; which produces central cholinergic activation restores blood pressure and sympathetic nervous system (SNS) activation during hemorrhage. Additional translational studies utilizing intravenous physostigmine have demonstrated not only improved blood pressure recovery following hemorrhage, but attenuated early tissue injury, partial restoration of the inflammatory response, and decreased mortality. These findings suggest that the observed increase in blood pressure is leading to improved tissue perfusion and maintenance of organ function. Taken together, these results are providing important pre-clinical data with potential for therapeutic development. Continuing studies are examining further the role of tissue hypoperfusion and altered central sympathetic signaling in the response to infection during the recovery period in addition to better characterizing the alterations in regional and organ blood flow as a result of our resuscitation model.